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[GB/GB]; 7 Ferrypoint, Undershore Road, Lymington,
Hampshire SO41 5TB (GB).

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(74) Agent: **WOODS, Geoffrey, Corlett**; J.A. Kemp & Co., 14
South Square, Gray's Inn, London WC1R 5JJ (GB).

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(71) Applicant (*for all designated States except US*): **UNIVERSITY OF SOUTHAMPTON** [GB/GB]; Highfield, Southampton SO17 1BJ (GB).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **SHUTE, Janis, Kay** [GB/GB]; 11 Widley Court Drive, Cosham, Portsmouth, Hampshire PO6 2QP (GB). **CARROLL, Mary, Patricia** [GB/GB]; Adult Cystic Fibrosis Unit, IDU Ward,, Southampton General Hospital, Tremona Road, Southampton SO16 6YD (GB). **CONWAY, Joy, Helen** [GB/GB]; Respiratory Cell Molecular Biology, University of Southampton, School of Medicine, Level D Centre Block, Southampton General Hospital, Tremona Road, Southampton SO16 6YD (GB). **HOCKEY, Peter, Morey**

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(54) Title: USE OF GLYCOSAMINOGLYCANS SUCH AS E.G. HEPARIN FOR THE TREATMENT OF RESPIRATORY DISORDERS SUCH AS COPD

(57) Abstract: A glycosaminoglycan or a physiologically acceptable salt thereof, which has an average molecule weight of from 8 to 40 kd can be used to treat chronic airflow limitation (COPD). The glycosaminoglycan or salt is typically inhaled



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USE OF GLYCOSAMINOGLYCANS SUCH AS E.G. HEPARIN FOR THE TREATMENT OF
RESPIRATORY DISORDERS SUCH AS COPD

Field of Invention

The present invention relates to treating chronic airflow limitation of the lung.

Background of Invention

Chronic airflow limitation (CAL) is a common disease worldwide. The majority of individuals with CAL are smokers, or former smokers, and this is thought to be one of the primary causes of the condition. However, not all sufferers of CAL are smokers and it is also believed that exposure to pollutants and/or certain inhaled chemicals may also be a contributory factor in the development of CAL.

At present CAL is treated only in its more developed stages using a variety of bronchodilators such as β_2 agonists and anti-cholinergic agents. A proportion of those with CAL respond to steroids, such as glucocorticosteroids. The existing treatments for CAL are generally regarded as only partially effective and are not thought to halt the progressive decline in lung function. An unfortunate factor is that CAL is only diagnosed in its later stages, at which point the decline in lung function is already well advanced.

The only proven disease modifying intervention at present recognised for CAL is the cessation of smoking. Stopping smoking may only slow the progressive decline in lung function found in CAL. It is thought that inflammation may continue even after smoking is stopped and that the existing inflammation, and in particular inflammatory cells present in the lung, may cause the release of inflammatory mediators that prolong or even amplify the existing inflammation. In addition, stopping smoking is often difficult for sufferers with some not stopping at all or resuming smoking later on. On average less than one third of patients are able to give up cigarettes even with support.

Summary of the Invention

The present invention is based on the unexpected finding that glycosaminoglycans with an average molecular weight of from 8 to 40 kd can be

used to treat CAL. The use of heparin is especially preferred. The use of administration by inhalation and/or intranasally is also particularly preferred.

Accordingly, the present invention provides for the use of a glycosaminoglycan or a physiologically acceptable salt thereof in the manufacture of a medicament for
5 treating chronic airflow limitation (CAL) wherein the said glycosaminoglycan or the salt has an average molecular weight of from 8 to 40 kilodaltons.

The present invention also provides:

- a method of treating CAL comprising administering to a subject suffering from CAL an effective amount of a glycosaminoglycan or a physiologically
10 acceptable salt thereof, where the glycosaminoglycan or salt has an average molecular weight of from 8 to 40 kilodaltons; and
- an agent for treating CAL comprising a glycosaminoglycan or a physiologically acceptable salt thereof, where the glycosaminoglycan or salt has an average molecular weight of from 8 to 40 kilodaltons.

15 The present invention further provides a kit comprising:

- a glycosaminoglycan, where the glycosaminoglycan or salt has an average molecular weight of from 8 to 40 kilodaltons;
- instructions for administering the glycosaminoglycan or salt to a subject suffering from CAL; and
20 - packaging.

Brief Description of the Figures

Figure 1: Effect of mucolytic agents on CAL sputum (n=3). As described in Example 1, CAL sputum was homogenised and mucolytic added on a 10 % v/w
25 basis. Mixing was performed using a 19-gauge needle. Carboxylate-modified fluorescent beads were added to sputum sample in a 1:1 ratio (v/w). After vortexing, 20 µl of sample was added to each upper well of a micro-boyden chamber. The upper and lower wells of the chamber were separated by an 8 µm polycarbonate filter, and the lower wells contained 25 µl PBS. The chamber was centrifuged for 5
30 mins at 1000 rpm to remove air bubbles prior to incubation at 37°C, 900 rpm, for 4 hours in the dark. Following incubation the chamber was dismantled and the

fluorescence of the solution in the lower wells measured. The results for PBS, heparin, DNase and dextran sulphate (a non-glycosaminoglycan control) are shown in the top panel. The results for a mixture of chondroitin sulphates A and C are shown in the bottom panel.

5 **Figure 2: Atomic Force Microscopy (AFM) imaging of DNA.** As described in Example 2, calf thymus DNA (0.1 mg/ml) was treated with heparin (0.1 mg/ml) and AFM was performed in air under ambient conditions using a TopoMetrix TMX2000 Scanning Probe Microscope (ThermoMicroscopes, Bicester, UK). Panel A shows untreated DNA and Panel B shows DNA treated with heparin.

10 **Figure 3: Atomic Force Microscopy (AFM) imaging of DNA.** Further Atomic Force Micrographs of DNA (100 µg/ml) treated with lower concentrations of heparin. Panel A shows the results for untreated DNA; Panel B for DNA treated with 0.1 µg/ml heparin; Panel C for DNA treated with 1 µg/ml heparin; and Panel D for DNA treated with 10 µg/ml heparin.

Detailed Description of the Invention

Chronic airflow limitation disorder (CAL) is a progressively debilitating condition which is often fatal. It is one of the most frequent causes of adult fatality. The present invention provides various medicaments, agents, methods and kits for
20 the treatment of CAL sufferers. These medicaments comprise a glycosaminoglycan, or a salt thereof. The glycosaminoglycan or salt will have an average molecular weight of from 8 to 40 kilodaltons.

Subjects treated

25 The medicaments and methods of the present invention are to be used to treat subjects suffering from, or at risk of developing, CAL. Typically, the subject will be a human but, as discussed further below, may be a vertebrate animal. CAL is a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal
30 inflammatory response of the lungs to noxious particles or gases. In particular, although not exclusively, CAL is associated with smoking.

For the purposes of the present invention CAL may be defined as a condition where there is a progressive decline in lung function, with the affected subject having an FEV₁ of less than 80% of that predicted for an individual of that age/race and/or height and/or who displays a FEV₁/FVC ratio of less than 70%. In an especially preferred embodiment, the subject to be treated will have an FEV₁ of less than 75% of that predicted.

Typically the reduction of FEV₁ is only partially reversible. In particular the reduction in FEV₁ is only partially reversible by treatment with bronchodilators such as, for example, β 2 adrenergic agonists and in particular salbutamol.

FEV₁ is defined as the maximal forced volume which can be expired in one second starting from maximum inspiration (European Resp. Journal, 1993; 6: Suppl. 16 and Coates, Lung Function, : Assessment and Applications In Medicine, 4th Edition, Oxford, Blackwell, 1969). It can be measured by standard techniques well known in the art i.e. by spirometry. The FEV₁ for an individual may be from 10 to 80% of that predicted. Typically, the FEV₁ of the subject will be from 10 to 75% of the predicted value. Preferably the subject may have a FEV₁ of from 60 to 75% the predicted value, more preferably from 40 to 60% of predicted and even more preferably a value below 40% of that predicted. The subject may have an FEV₁ of less than 70%, preferably less than 60%, more preferably less than 50% and even more preferably less than 40% of that predicted. As discussed further below, the subject will typically have a history of exposure to pollutants or chemicals and in many cases will be, or have been, a tobacco smoker.

The FEV₁ value for the subject will normally be measured against predicted values and adjusted for age/sex/race and/or height. Predicted values may be those taken from Coates (*supra*). The expected value, which the value obtained for the subject may be compared to, may be the average expected value for smokers, or non-smokers, or both groups combined, preferably the expected value will be that for non-smokers not suffering from CAL (Coates, *supra*).

The reduction in FEV₁ in the subject will only be partially reversible and in particular only be partially reversible on administration of a bronchodilator. Thus, for example, an increase in FEV₁ over the base-line value for the subject (i.e. that

prior to administration of the bronchodilator) of more than 15%, preferably more than 20% and even more preferably over 25% will be regarded as reversibility. The increase may begin from 5 to 30, preferably from 10 to 25, more preferably over a period of from 15 to 20 minutes after the administration of the bronchodilator.

5. Preferably the increases will begin from 15 minutes after the administration of the bronchodilator. The increases persist typically from 3 to 6 hours, preferably from 4 to 5 hours and more preferably 4 hours. Typically the bronchodilator used in assessing reversability will be a β_2 adrenergic agonist such as salbutamol, or ipratropium. In one embodiment of the invention the reduction in FEV₁ may be
- 10 totally, or almost totally refractory to treatment with bronchodilators.

The subject may also show similar minimal increases in FEV₁ with steroid drugs such as Becotide™ or Prednisolone™ although typically response to such agents will not be used to define reversibility. The increases will also occur over a longer time period such as after 2 to 3 days and, if the steroid drugs are continually

15 administered, persist. If steroids are stopped the improvement may persist for from 12 to 48 hours, or for days, weeks or even months, such as from six hours to six weeks, preferably from 1 day to 3 weeks.

Tests to assess reversibility of reduction of FEV₁ will typically be performed when the subject is clinically stable and free from infection. The subject should not

20 have taken, or have had administered to them, inhaled short-acting bronchodilators in the previous six hours, long-acting β agonists in the previous 12 hours or sustained release theophyllines in the preceding 24 hours.

Spirometric values should typically be measured before and after an adequate dose of inhaled bronchodilator is given to the subject. The dose should preferably be

25 selected to be high on the dose/response curve and usually will be given by nebuliser to be certain it has been inhaled. A similar dose may be given with multiple inhalations from a metered dose inhaler and large volume spacer, but this is less preferred. A typical dosage/measurements protocol for a human subject would be:

- before and 15 minutes after 2.5 to 5mg nebulised salbutamol or 5 to 10
- 30 mg terbutaline;
- before and 30 minutes after 500 μ g nebulised ipratropium bromide; or

- before and 30 minutes after both in combination.

Equivalent protocols may be used for non-human subjects.

The FVC of the subject may also be measured in the diagnosis of CAL. The ratio of FEV_1 to FVC can be used in the diagnosis of CAL. Subjects to be treated will typically have an FEV_1 /FVC value of less than 70%. The ratio of FEV_1 /FVC may be below 65%, preferably below 60%, more preferably below 55% and even more preferably below 55%. In an especially preferred embodiment, a subject will have a FEV_1 /FVC of below 70% and also have a FEV_1 value of 80% or less of that predicted.

10 FVC (forced vital capacity) corresponds to the maximal volume of air forcibly exhaled from the point of maximal inhalation and can be measured using standard spirometry. In particular, the above specified values for FEV_1 /FVC will be those after administration of a bronchodilator as outlined above. The reduction in FEV_1 /FVC will typically show the same lack of reversibility as FEV_1 .

15 Spirometric assessment is the most preferred method for diagnosing CAL and hence method for identification of subjects who may be treated. Accordingly, in an especially preferred embodiment of the invention spirometric assessment will be used in the diagnosis of a subject who has CAL and hence is treatable using the invention. In addition, the symptoms displayed by the subject may also be assessed to help confirm a diagnosis of CAL. Typically diagnosis will involve spirometric
20 assessment in combination with assessment of the symptoms of a subject as well as elucidating whether the subject has a history of exposure to risk factors. In some situations spirometric assessment may not be possible, particularly in situations where resources are limited, and CAL will be diagnosed by alternative means such as
25 by looking for the symptoms of CAL listed herein and a history of exposure to risk factors for CAL. Although chest X-rays are not typically indicative of whether or not a subject has CAL, they may be used to diagnose other respiratory disorders, such as TB, and hence rule out CAL.

The subject will show, or have previously shown, an accelerated rate of decline
30 of lung function compared to the average expected for an equivalent individual not suffering from CAL and in particular for an equivalent non-smoking individual. The

subject may display shortness of breath and in particular may do so after physical exertion such as on exercise. Typically, this will not be induced by exposure to an allergen. The subject may also show increased incidence of bacterial or viral infection and this may exacerbate the condition.

5 The individual may show a rate of decrease in FEV_1 one, two, three, four or more times greater than the average annual value expected for an equivalent individual not suffering from CAL. For instance a subject over thirty may show an annual reduction of from 50 to 100, preferably from 50 to 80 and more preferably from 60 to 70 ml FEV_1 /yr compared to a reduction of from 10 to 40 and typically of
10 20 to 40 ml of FEV_1 /yr in the equivalent non-smoker. These values may also apply to non smoking sufferers of CAL such as where the disorder is caused by pollutants.

Subjects with CAL may display one or more, and sometimes all, of cough, increased sputum production, dyspnea, and/or a history of exposure to risk factors for the disease. In the case of cough, increased sputum and dyspnea these may have
15 been present for extended periods of time such as at least a month, preferably six months, more preferably at least a year and still more preferably for at least two years. Chronic cough and sputum production often precede the development of CAL and may be indicative of individuals for which the invention can be used prophylactically to prevent the development of CAL.

20 The subject may have a history of exposure to pollutants and/or chemicals and in particular to those in a form which can be inhaled. In particular, the subject may be a tobacco smoker or a former tobacco smoker. Typically, the subject may be, or have been, a heavy smoker, smoking from 10 to 100, preferably from 20 to 60, more preferably from 25 to 50 and even more preferably from 30 to 40 cigarettes per
25 day. The subject may have done so for several years such as from 5 to 50, preferably from 10 to 40, more preferably from 15 to 30 and even more preferably from 20 to 25 years. The subject may typically have a smoking history of from 1 to 20, preferably 21 to 40, more preferably 41 to 60 pack years. They may have a smoking history, for example, of from 1 to 50, preferably from 10 to 40, more preferably from
30 20 to 30 pack years.

The individual may have been, or be, a pipe or cigar smoker. The individual

may have chewed tobacco or products containing tobacco. In some cases the individual may have been passively exposed to tobacco smoke rather than smoke themselves. Thus the subject may, for example, have been cumulatively exposed to passive tobacco smoke for long periods due to their work, leisure and/or home environments. The subject may use inhaled drugs such as, for example cannabis or other drugs commonly mixed with tobacco before smoking.

The subject will typically be a mature adult. For example, the subject may be from 21 to 85, preferably from 25 to 70, more preferably from 30 to 60 and even more preferably from 40 to 50 years of age. The onset of any of, or a particular, symptom mentioned herein, will typically have been in adulthood. For example, the subject may have been at least 20, more preferably at least 25, still more preferably at least 30 and even more preferably at least 35 years of age before they experienced a particular symptom. In particular, the symptoms associated with more advanced stages of CAL, such as any of those mentioned herein, may have their onset at such later stages of life. Subjects with a genetic predisposition to developing CAL, such as those with α_1 -antitrypsin deficiency, may develop CAL earlier. For example, they may display one or more, or a particular, symptom at from 10 to 21, preferably from 12 to 18, or more preferably from 14 to 16 years of age. Alternatively, they may first show the symptom at any of the age ranges mentioned herein. The subject may have been diagnosed at any of the ages, or within any of the age ranges, specified herein.

The subject may have additionally been, or alternatively been, exposed to other chemical or environmental pollutants. The subject may have been exposed to occupational dusts and chemicals (vapours, irritants and fumes) and/or indoor or outdoor air pollution. The subject may have a history of exposure to particulate matter, irritants, organic dusts and sensitising agents. The subject may work, or have worked, in an environment which exposes them to chemicals and/or pollutants. Thus, for example, the subject may be a factory worker or miner such as a coal miner. The subject may cook in kitchens with poorly ventilated cooking stoves such as in the presence of kerosene cookers. This, in particular, may be the case in developing countries such as Africa. The subject may work in the construction industry. The subject may have been exposed to high levels of pollution, such as exhaust emissions

from vehicles or other engines. The subject may have been exposed to smog or sulphur dioxide containing emissions. The individual may live in the city centre. The individual may live in close proximity to heavy industry.

The subject may have a genetic predisposition to developing CAL and may display a family history of the condition. For example, the subject may have α_1 -antitrypsin deficiency and hence be predisposed to developing CAL. Subjects at risk of developing CAL may have had low birth weights and/or a history of exposure to pollutants in the womb or in early life. The mother of the subject may be a smoker and may have continued to smoke during pregnancy. The subject may have had a history of severe childhood respiratory infection. Reduced maximal attained lung function, as measured by spirometry, may identify individuals who are at increased risk of developing CAL.

The subject may have an early stage of CAL in which the symptoms are generally moderate and may have periods of normality or of at least reduced symptoms. Alternatively, the subject may have a more developed stage of CAL in which the symptoms, and particular the reduction in FEV_1 , is more pronounced. Preferably, the methods and medicaments of the invention are used, or administered at, an early stage of CAL so that they can arrest, slow or regress the increased rate in FEV_1 decline at as early a stage as possible.

CAL is typically a progressive disorder with the severity of CAL and the extent to which it has an impact on the sufferer increasing over time. Thus there may be a progressive manifestation in the symptoms of the disorder. Chronic cough is usually the first symptom to develop. It may initially be intermittent, but later may be present every day. The cough will typically be present throughout the day, rather than just at night and in the morning. In some cases, significant airflow limitation may develop without the presence of a cough. Small amounts of tenacious sputum are commonly raised after coughing bouts.

As the disease progresses the subject may experience dyspnea. The onset of dyspnea will often be one of the reasons why a human subject will first consult a physician as it can be debilitating and also induce anxiety. As the lung function of the subject deteriorates further, breathlessness becomes more intrusive. The subject

may display wheezing and chest tightness. The dyspnea may become worse during or after exercise. Respiratory infections may also exacerbate the condition and cause increased dyspnea. Human subjects may indicate that the dyspnea progressively impairs their ability to carry out physical labour and to exert themselves.

- 5 CAL sufferers are sometimes split into categories which reflect the stage and severity of the disease. This can help define the needs of a particular subject and what course of treatment should be administered. In one classification (GOLD Executive Summary, supra) subjects are split into the following categories:

10 Category 0 – At risk

Lung function, as measured by spirometry, is normal.

Chronic symptoms (cough, sputum, production).

Typically, human subjects will be unaware of abnormal lung function.

15 Category I: Mild CAL

Mild airflow limitation.

$FEV_1/FVC < 70\%$.

FEV_1 greater than, or equal to, 80% of that predicted.

With or without chronic symptoms (cough, sputum, production).

- 20 Human subjects may be unaware that their lung function is abnormal.

Category II: Moderate CAL

Worsening airflow limitation.

$FEV_1/FVC < 70\%$.

- 25 FEV_1 from 30 to 80% of that predicted (category can be subdivided into: IIA - FEV_1 from 50 to 80% of that predicted; and IIB - FEV_1 from 30 to 50% of that predicted).

With, or without, chronic symptoms (cough, sputum, production, dyspnea). The symptoms may typically become more pronounced on exertion. Human subjects are likely to be aware of the condition and have sort medical advice.

Category III: Severe CAL

Severe airflow limitation.

$FEV_1/FVC < 70\%$

- 5 FEV_1 less than 30% predicted or alternatively an FEV_1 less than 50% of that predicted in conjunction with respiratory failure or clinical signs of right heart failure (respiratory failure: arterial partial pressure of oxygen (PaO_2) less than 8.0 kPa (60 mm Hg) with or without arterial partial pressure of CO_2 ($PaCO_2$) greater than 6.7 kPa (50 mm Hg) while breathing air at sea level).

10

The subject to be treated using the invention may fall within any of the above categories. In particular, the subject may be one in categories I to III, preferably in categories II to III, and even more preferably in category III. The invention also encompasses the treatment of individuals at risk of CAL (category 0) and with an
15 early stage of the disorder (category I).

CAL is typically characterized by chronic inflammation throughout the airways, parenchyma, and pulmonary vasculature. Macrophages, T lymphocytes (predominately $CD8^+$) and neutrophils may be present in increased levels in various parts of the lung. Typically, the condition will be characterised by the presence of
20 large numbers of infiltrating neutrophils rather than eosinophils. Activated inflammatory cells release a variety of mediators, including leukotriene B4 (LTB4), interleukin 8 (IL-8), tumor necrosis factor (TNF) and others, and any of these may be present in elevated levels in a subject in comparison to healthy individuals who do not have CAL. In particular those mediators capable of causing damage to lung
25 structure and/or sustaining neutrophilic inflammation will be present in elevated levels. The subject may also display an imbalance of proteinases and anti-proteinases in the lung and also elevated oxidative stress.

The subject may also display increased inflammation. There may be elevated levels of inflammatory mediators present in the airways of the subject. These may
30 include proteases such as matrix metallo proteases (MMPs), cathepsins and elastase. They may also include myelo-peroxidase, activated complement, MCP-1, interferons

(such as, in particular, IFN- γ) and interleukins such as, in particular, IL-12.

Typically, IL-8 and in particular elevated IL-8 levels will be present.

The lungs of the subject, or regions of them, may have increased numbers of inflammatory cells in comparison to those of an equivalent individual without CAL.

5 In particular, there may be an influx of neutrophils and/or CD8⁺ T cells into the airways and lung. There may also be changes in the bronchial and pulmonary vasculature, such as upregulation of adhesion molecules involved in leukocyte extravasation or increased expression of inflammatory cytokines which can recruit inflammatory cells such as neutrophils, monocytes and T cells.

10 The neutrophils may be primed or in an activated state. For example, they may show an increase in expression of various adhesion molecules and receptors. There may also be an elevated level of neutrophil degranulation and hence of neutrophil proteins in the lungs. These may include proteins such as proteases (including cathepsins and neutrophil elastase), other hydrolytic enzymes and various
15 antibacterial proteins. There may also be an elevation in the level of reactive oxygen species released by the neutrophils. These changes may be detectable in the subject by examining samples such as sputum or lavages from the subject. There may also be elevated levels of other granulocytes present in the inflamed airway and lung such as basophils and/or eosinophils, but typically these will not be the predominate species
20 of granulocytes.

In some of the subjects there will be permanent destructive enlargement of the airspaces distal to the terminal bronchioles without obvious fibrosis. There may be chronic hypersecretion of mucus. Thus mucus secretion may, for example, be increased one, two, three or four times. The viscosity of the mucus may also be
25 increased and this may be at least partially due to the presence of polymers such as DNA and actin. The chronic bronchial secretions may in some cases be such that they are enough to cause expectoration, occurring on most days for a minimum of three months of the year for two consecutive years.

In some cases, there may be extracellular DNA, and in particular endogenous
30 extracellular DNA, present in the lungs of the subject, including in the airways of the lungs. The DNA may be extracellular DNA originating from the cells of the body of

the subject. Typically this DNA will originate from the nucleus and hence be genomic DNA. The DNA may originate from infiltrating inflammatory cells.

In cases where extracellular DNA is responsible for, or contributes to, an increase in mucus viscosity in the subject, it will typically be of high molecular weight. Thus it may be, for example, that it has an average fragment size, fragment size range or predominate fragment size in the range of from 100 bases to 1 megabase, preferably from 1 kb to 500 kb, more preferably from 5 kb to 250 kb in length, still more preferably from 25 kb to 100 kb and even more preferably from 25 kb to 50 kb in length. The DNA will typically be wholly, or partially free of, histones or will comprise regions which lack histones. It may be bound by cationic factors or factors with cationic groups such as inflammatory mediators and/or proteases. Thus for example it may be complexed or associated with elastase, cathepsins and/or IL-8. The presence of the DNA will typically increase the viscosity of the mucus, or other secretion, that it is present in.

Typically, the presence of DNA may increase the viscosity of the mucus by from 10 to 5000%, preferably from 50 to 2500%, more preferably from 100 to 1000%, still more preferably from 200 to 800% and even more preferably from 400 to 600%. The viscosity of the solution may typically be doubled, tripled, quadrupled or increased by five fold or more in comparison to the viscosity of the solution in the absence of the DNA. The viscosity of the DNA may be such that it may compromise a function in the subject such as, for example, lung function. It may contribute to airflow limitation. It may promote the occurrence of infections.

Pathological changes characteristic of CAL may typically be found in the central airways, peripheral airways, lung parenchyma and/or pulmonary vasculature of the subject. In the central airways (the trachea, bronchi and bronchioles greater than 2 to 4 mm in internal diameter) infiltrating inflammatory cells may be present in the surface epithelium. Enlarged mucus secreting glands and an increase in the number of goblet cells may also be seen and are typically associated with mucus hypersecretion. In the peripheral airways (small bronchi and bronchioles that have an internal diameter of less than 2 mm) chronic inflammation may lead to repeated cycles of injury and repair of the airway wall. The repair process typically results in

remodeling of the airway wall, with increasing collagen content and scar tissue formation, that narrows the lumen and produces fixed airways obstruction.

Destruction of the lung parenchyma in subjects with CAL typically occurs as centrilobular emphysema. This involves dilatation and destruction of the respiratory
5 bronchioles. These lesions occur more frequently in the upper lung regions in milder cases, but in advanced disease they may appear diffusely throughout the entire lung and also involve destruction of the pulmonary capillary bed. An imbalance of endogenous proteinases and antiproteinases in the lung, which may be due to genetic factors or the action of inflammatory cells and mediators, may be a major mechanism
10 in the emphysematous lung destruction. Oxidative stress, another consequence of inflammation, may also play a part in the destruction.

The subject may display pulmonary vascular changes, with thickening of the vessel wall that begins early on in the development of the disease. Thickening of the intima is the first structural change, followed by an increase in smooth muscle and
15 the infiltration of the vessel wall by inflammatory cells. As the severity of the disease increases, greater amounts of smooth muscle, collagens and proteoglycans may increase the thickness of the vessel wall further.

The lungs of the subject may display a number of physiological changes characteristic of CAL, including mucus hypersecretion, ciliary dysfunction, airflow
20 limitation, pulmonary hyperinflation, gas exchange abnormalities, pulmonary hypertension, and cor pulmonale. Often such abnormalities will manifest themselves in that order, although they may not necessarily do so. Mucus hypersecretion and ciliary dysfunction may contribute to chronic cough and sputum production. These symptoms can be present for many years before other symptoms or physiological
25 abnormalities develop and may be used to detect individuals who have a very early stage of CAL or are at risk of the disorder.

In more advanced stages of CAL, peripheral airways obstruction, parenchymal destruction, and pulmonary vascular abnormalities reduce the capacity of the lung for gas exchange, producing hypoxemia and, eventually, hypercapnia.
30 Pulmonary hypertension, which develops late in the course of CAL when the disorder has reached its severe stage, is the major cardiovascular complication of

CAL and may be associated with the development of cor pulmonale and a poor prognosis.

The subject will be a vertebrate animal and preferably will be a mammal. Typically the subject will be human. However, the invention also encompasses the treatment of animals with conditions which are the same as, or equivalent to, CAL. Thus the animal condition may have either an underlying pathology similar to, or the same as, that of CAL in humans. The animal may have one or more similar symptoms or characteristics of human CAL and in particular one or more of those listed above. Preferably, the animal will suffer from a condition which falls within the definition of CAL given above.

In cases where the subject is not human it may be a domestic animal or an agriculturally important animal. The animal may, for example, be a sheep, pig, cow, bull, poultry bird or other commercially farmed animal. In particular, the animal may be a cow or bull and preferably is a dairy cow. The animal may be a domestic pet such as a dog, cat, bird, or rodent. In a preferred embodiment the animal may be a cat or other feline animal. The animal may be a monkey such as a non-human primate. For example, the primate may be a chimpanzee, gorilla, or orangutan. In a preferred embodiment of the invention the animal may be a horse and, for example, may be a racehorse. The animal may be a sports animal.

Some of the symptoms of CAL are displayed by sufferers of other diseases. For example, a number of respiratory disorders other than CAL compromise lung function. However, these diseases may be distinguished from CAL by a full assessment of the subject and preferably by applying the relevant available guidelines for the diagnosis of CAL. The British Thoracic Society have published a set of Guidelines (Thorax 1997, 52 (Suppl. 5): S1-28) and in an especially preferred embodiment a subject to be treated will fall within the definition of the disorder provided by these Guidelines. In addition, the Global initiative for chronic Obstructive Lung Disease (GOLD) has published an Executive Summary (2001) outlining a strategy for the diagnosis, management and prevention of the disorder. In a preferred embodiment the subjects to be treated will fall within the definition of the disorder provided by the Executive Summary.

CAL does not include cystic fibrosis (CF). Thus typically a subject who can be treated using the invention will have a least one functional copy of the CFTR gene (unless they have been unfortunate enough to develop both CF and CAL). CAL typically has a much later onset than Cystic Fibrosis, where a sufferer is born with the defect. In CAL the decline in lung function and the onset of symptoms will occur progressively over time. In CF the decline in lung function will have a more immediate onset, earlier on in life. A subject who has CAL will often be in their thirties, forties or even fifties before they become aware that they are suffering from the disorder. The major exception to the later onset of CAL, is in those sufferers who have α_1 anti-trypsin deficiency. Such subjects will display an earlier onset of CAL, such as in their teens or twenties when CAL is diagnosed, as they will be born with a genetic condition predisposing them to CAL. Subjects who have α_1 antitrypsin deficiency and CF can readily be distinguished from each other by biochemical and genetic tests to identify the nature of the disorder.

Glycosaminoglycans

The medicaments and methods of the invention employ glycosaminoglycans. Glycosaminoglycans are linear heteropolysaccharides possessing characteristic disaccharide repeat sequences that are typically highly N- and O-sulphated at D-glucosamine, galactosamine and uronic acid residues. These sulphate moieties introduce a high degree of negative charge along the glycosaminoglycan polymer chain and add to the heterogeneity of these macromolecules.

Any suitable glycosaminoglycan may be employed in the invention.

Glycosaminoglycans and glycosaminoglycan salts suitable for use in the present invention will have an average molecular weight of from 8 to 40 kd, preferably from 10 to 30 kd, more preferably from 12 to 20 kd. In particular, the glycosaminoglycan or salt may have an average molecular weight of from 14 to 18kd, preferably from 15 to 17 kd and more preferably from 16 to 17 kd. In some embodiments all, or substantially all, of the glycosaminoglycan molecules or glycosaminoglycan salt molecules will have a molecular weight falling within the ranges specified above. Thus from 50 to 100%, preferably from 75 to 100%, more preferably from 90 to

100%, still more preferably 95 to 100% of the molecules may have such a molecular weight. In some cases at least 95%, preferably 97.5%, more preferably 99%, still more preferably 99.5% and even more preferably 99.9% may have a molecular weight falling within the range. The glycosaminoglycan or salt may be present in a
5 range of molecular weight sizes and typically the most commonly occurring molecular weight size will fall within one of the above specified molecular weight ranges.

Preferably, the glycosaminoglycan employed in the invention will be any of chondroitin sulphates A to E, heparin, heparin sulfate, heparan, heparan sulphate,
10 hyaluronic acid, keratan sulphate, a derivative of any thereof or a mixture or any two thereof. Chondroitin sulphate B is sometimes referred to as dermatan sulphate. In a more preferred embodiment of the invention the glycosaminoglycan will be any of chondroitin sulphates A, C, D or E, heparin, heparin sulfate, heparan, heparan sulphate, hyaluronic acid, keratan sulphate, a derivative of any thereof or a mixture of
15 any two thereof. In a particularly preferred embodiment the glycosaminoglycan will be chondroitin sulphate A, chondroitin sulphate C, heparin, heparin sulfate, heparan, heparan sulphate, a derivative of any thereof or a mixture of any two thereof. More preferably, the glycosaminoglycan will be chondroitin sulphate A, chondroitin sulphate C, heparin, a derivative of any thereof or a mixture of any two thereof. In an
20 even more preferred embodiment of the invention the glycoaminoglycan will be heparin or a derivative thereof. In some embodiments of the invention the glycosaminoglycan employed will be a mixture of more than two glycosaminoglycans from one of the above mentioned groups, such as a mixture of three, four or five of the glycosaminoglycans.

25 In embodiments of the invention where a mixture of two glycosaminoglycans is employed the two may, for example, be present in the ratio 1:1, 1:2, 1:4, 1:10 or 1:100. The ratio may be 90:10, 80:20, 70:30, or 60:40. Any suitable ratio may be employed and either glycosaminoglycan may be at the higher concentration. The ratio may be the same as the ratio in which the two are isolated when they are
30 recovered from a common tissue using standard techniques. In a preferred embodiment of the invention a mixture of chondroitins A and C will be employed

and in particular at a ration of 80:20, preferably 75:25 and even more preferably 70:30 with chondroitin sulphate A being present at the higher level.

Typically, the glycosaminoglycan will not have been subjected to fragmentation to reduce its molecular weight. Usually, the glycosaminoglycan will not have been subjected to depolymerisation, such as by chemical or enzymatic means, to reduce its molecular weight. The average number of saccharide units in the polysaccharide chains of the glycosaminoglycan may typically be from 18 to 100, preferably from 30 to 80, more preferably from 40 to 60 and still more preferably from 5 to 60 units.

The glycosaminoglycan may be any suitable commercially available glycosaminoglycan and may, for example, be an unfractionated glycosaminoglycan. The glycosaminoglycan will have typically been isolated from a natural sources such as from an animal. In some cases, the glycosaminoglycan may have been synthesised rather than be a naturally occurring molecule.

In some cases, the glycosaminoglycan may have been isolated from an animal, and in particular from animal tissues such as those of pigs or cattle. The glycosaminoglycan may have been obtained from tissues such as the lung, liver, or gut of an animal and in particular from beef lung or pork intestinal mucosa. The glycosaminoglycan may have been obtained from the skin of such an organism.

In some embodiments, the glycosaminoglycan may have been isolated from a cartilagenous fish or other sea or freshwater organism. In some cases the glycosaminoglycan may have been isolated from a shark or squid and in particular from the cartilage of such an organism. The glycosaminoglycan may have been isolated from a sturgeon and in particular from a sturgeon notochord.

One of the specific glycosaminoglycans mentioned above may have been modified to generate a derivative of the glycosaminoglycans. Thus such derivatives may be used in the invention as long as they retain therapeutic activity in treating CAL and in particular are capable of eliminating, reducing, ameliorating or managing one or more of the symptoms and manifestations of CAL discussed herein. Thus in the case of heparin, the heparin may have been subjected to O-desulphation such as at least at the 2-O and 3-O positions. The same or equivalent modifications may be

made to other glycosaminoglycans to generate derivatives for use in the present invention.

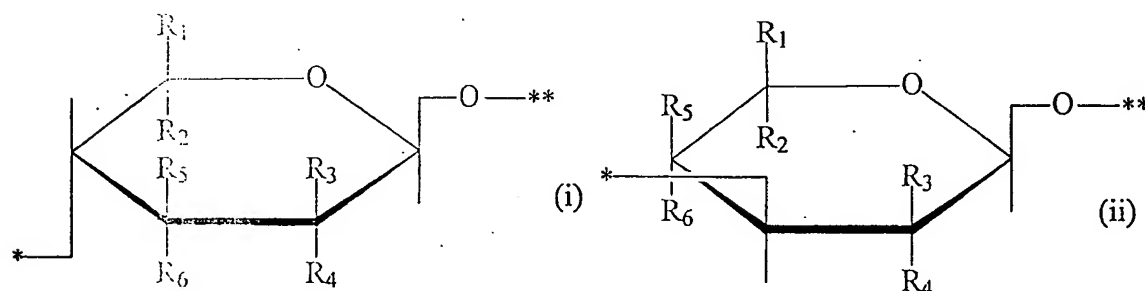
The glycosaminoglycan may have been subjected to acetylation, deacetylation, oxidation and/or decarboxylation such as, for example, periodate oxidation to generate a derivative. Heparinoids may be used in the invention.

Typically, the active compound used in the present invention comprises is a glycosaminoglycan or a physiologically acceptable salt thereof comprising repeating disaccharide units of general formula (1)



wherein:

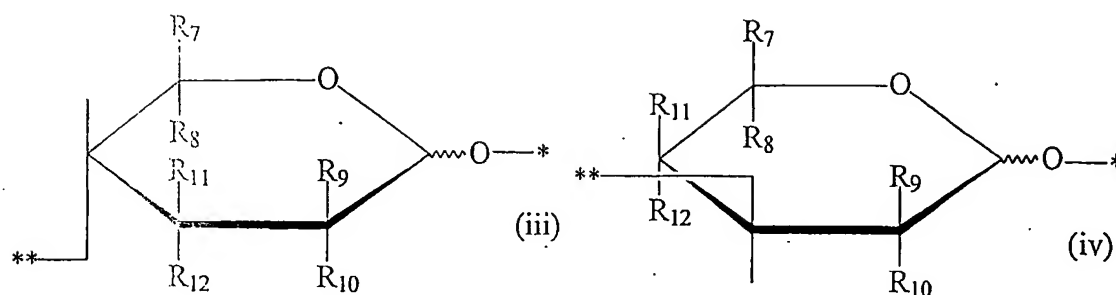
each A is the same or different and represents a moiety of formula (i) or (ii)



wherein:

- 15 - one of R_1 and R_2 is hydrogen, and the other is $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$ or $-\text{CH}_2\text{OR}$ wherein R is hydrogen or $-\text{SO}_3\text{H}$;
- one of R_3 and R_4 is hydrogen, and the other is $-\text{OR}$ wherein R is hydrogen or $-\text{SO}_3\text{H}$;
- one of R_5 and R_6 is hydrogen, and the other is $-\text{OH}$;
- 20 - * represents a direct bond to an adjacent hydrogen atom or B moiety; and
- ** represents a direct bond to an adjacent B moiety;

each B is the same or different and represents a moiety of formula (iii) or (iv);



wherein:

- one of R_7 and R_8 is hydrogen and the other is $-\text{CH}_2\text{OH}$ or $-\text{CH}_2\text{OSO}_3\text{H}$;
- one of R_9 and R_{10} is hydrogen and the other is $-\text{NHAc}$, $-\text{NH}_2$ or $-\text{NHSO}_3\text{H}$;
- 5 - one of R_{11} and R_{12} is hydrogen and the other is $-\text{OH}$ or $-\text{OSO}_3\text{H}$;
- * represents a direct bond to a hydrogen atom or an adjacent A moiety;
- ** represents a direct bond to an adjacent A moiety; and
- indicates a bond in either stereochemical orientation;

or a physiologically acceptable salt thereof.

10 The formulae herein adopt standard practice in depicting sugars. According to this practice, the formulae include vertical lines through each of the cyclic carbon atoms. This does not, of course, mean that methyl groups are attached at each position, or that methylene groups are present as part of the link between adjacent cyclic moieties.

15 Preferably, each A moiety in the glycosaminoglycan of general formula (1) is the same. Preferably, each B moiety in the glycosaminoglycan of general formula (1) is the same.

Preferably, each A moiety in the glycosaminoglycan of general formula (1) is a moiety of general formula (i).

20 Typically, one of R_1 and R_2 is hydrogen, and the other represents $-\text{CO}_2\text{H}$ or $-\text{CH}_2\text{OR}$, wherein R is hydrogen or $-\text{SO}_3\text{H}$. Preferably, one of R_1 and R_2 is hydrogen and the other represents $-\text{CO}_2\text{H}$.

Typically, R_3 is hydrogen and R_4 is $-\text{OR}$, wherein R represents hydrogen or $-\text{SO}_3\text{H}$.

25 Typically R_5 is $-\text{OH}$ and R_6 is hydrogen.

Typically each A is the same or different and represents a moiety of formula

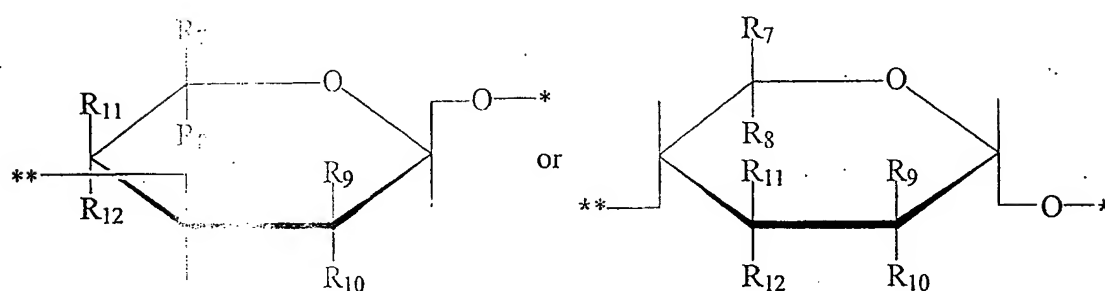
(i).

Typically R_7 is $-\text{CH}_2\text{OH}$ or $-\text{CH}_2\text{OSO}_3\text{H}$ and R_8 is hydrogen.

Typically R_9 is hydrogen and R_{10} is $-\text{NHAc}$, $-\text{NH}_2$ or $-\text{NHSO}_3\text{H}$.

5 Typically R_{11} is $-\text{OSO}_3\text{H}$ or $-\text{OH}$ and R_{12} is hydrogen.

Typically each B is the same or different and represents



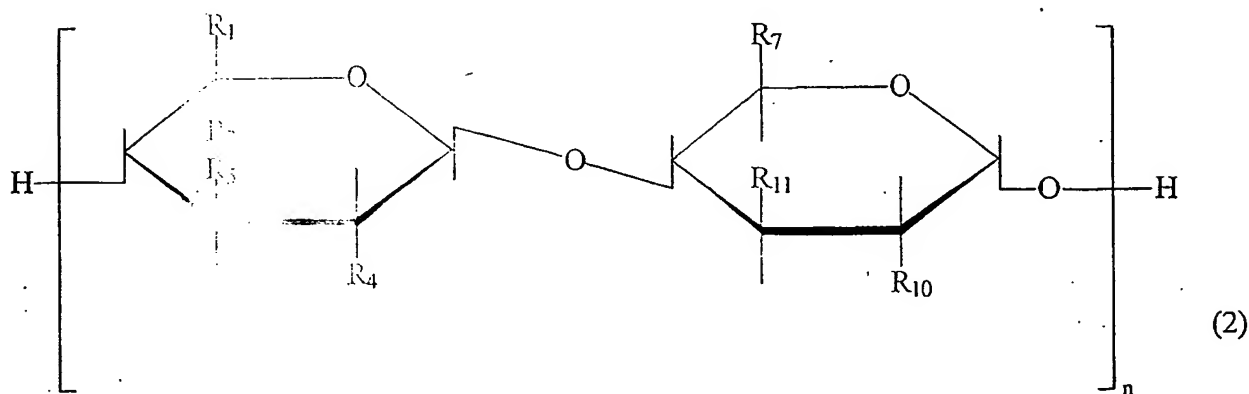
10 wherein R_7 , R_8 , R_9 , R_{10} , R_{11} , R_{12} , * and ** are as described above.

Preferably R_1 is not hydrogen in an A moiety which is adjacent to a moiety

(iv) in which R_{12} is hydrogen.

Typically the glycosaminoglycan of general formula (1) is a
glycosaminoglycan of general formula (2)

15

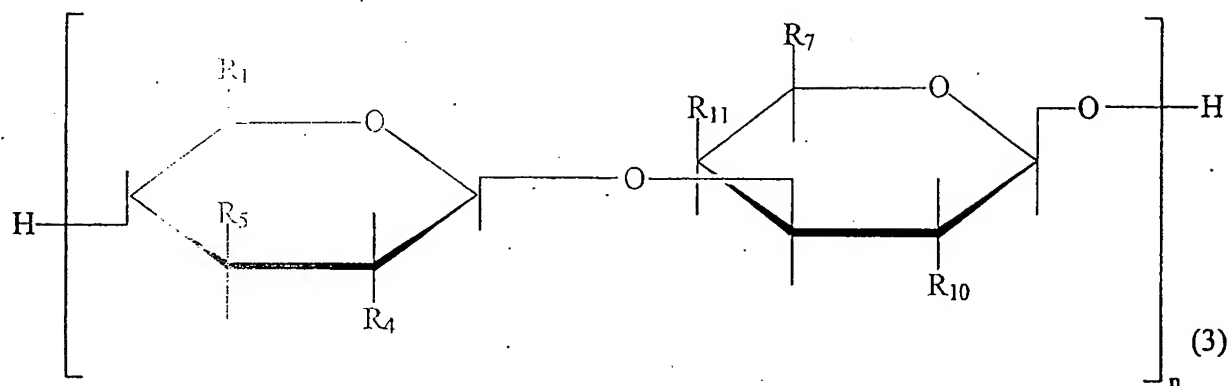


wherein:

- or if R_1 and R_2 is hydrogen and the other is $-\text{CO}_2\text{H}$;

- R_1 is $-\text{OH}$ or $-\text{OSO}_3\text{H}$;
- R_2 is $-\text{OH}$;
- R_3 is $-\text{CH}_2\text{OH}$ or $-\text{CH}_2\text{OSO}_3\text{H}$;
- R_{10} is $-\text{NH}_2$, $-\text{NHSO}_3\text{H}$ or $-\text{NHAc}$; and
- 5 - R_{11} is $-\text{OSO}_3\text{H}$ or $-\text{OH}$.

Typically the glycosaminoglycan of general formula (1) is a
glycosaminoglycan of general formula (3)



wherein:

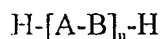
- 10 - R_1 is $-\text{CO}_2\text{H}$;
- R_2 is $-\text{OH}$;
- R_3 is $-\text{OH}$;
- R_4 is $-\text{CH}_2\text{OH}$ or $-\text{CH}_2\text{OSO}_3\text{H}$;
- R_{10} is $-\text{NHAc}$; and
- 15 - R_{11} is $-\text{OH}$ or $-\text{OSO}_3\text{H}$.

Any suitable physiologically acceptable glycosaminoglycan salt may be employed in the invention and in particular a metallic salt, for example a sodium salt, an alkali metal or an alkaline earth metal salt. Other salts include calcium, lithium and zinc salts. Ammonium salts may also be used. The salt may be a sodium
20 glycosaminoglycanate or glycosaminoglycan sulphate. Salts of derivatives of specific glycosaminoglycans mentioned herein may also be used in the invention. In the present application where mention of a glycosaminoglycan is made, such mention also includes physiologically acceptable salts thereof.

Typically a physiologically acceptable salt is a salt with a physiologically acceptable acid or base. Preferred salts are salts with physiologically acceptable bases. Typically, such salts are compounds wherein the acidic hydrogen atom of a -CO₂H and/or -OSO₃H group is replaced with a cation, for example an alkali metal (e.g. sodium or potassium) or alkali earth metal (e.g. calcium or magnesium) cation. Such salts can be prepared, for example, by reaction with an appropriate hydroxide.

The number of disaccharide units present in the glycosaminoglycan or salt thereof employed in the invention will be such that the molecular weight of the glycosaminoglycan or salt is from 8 to 40 kd, preferably from 10 to 30 kd, more preferably from 12 to 20 kd. In particular, it may be such that the glycosaminoglycan has a molecular weight of from 14 to 18 kd, preferably from 15 to 17 kd and more preferably from 16 to 17 kd. The number of disaccharide units present in the glycosaminoglycan may be represented by the number n, where n is any integer such that the glycosaminoglycan has a molecular weight falling within any of the above mentioned molecular weight ranges.

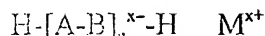
Thus the glycosaminoglycan or salt employed may be represented by the general formula:



wherein A-B is any of the disaccharide units mentioned above and n is an integer such that the glycosaminoglycan or salt thereof has a molecular weight falling within the above specified molecular weight ranges. The value of n may be, for example, from 30 to 55 and more preferably from 35 to 50.

The glycosaminoglycan employed in the invention will typically comprise more than one length chain. Hence n for some of the glycosaminoglycan chains present may be a lower or higher integer than an integer which, on its own, would give a chain of molecular weight size falling within one of the above specified ranges. Thus the average value of n of the glycosaminoglycans present in the medicaments of the invention may be any of the values specified for n herein and in particular a value of n which gives a molecular weight glycosaminoglycan or salt falling within one of the molecular weight ranges specified herein.

Particularly preferred salts for use in the invention are salts of formula:



wherein $x \leq 4n$ and M represents a physiologically acceptable cation or a mixture thereof. Most preferably, $x \leq 2n$.

In a particularly preferred embodiment of the invention the
 5 glycosaminoglycan employed will be a heparin, derivative thereof or a physiologically acceptable salt thereof. Heparin is a naturally occurring mucopolysaccharide present in a variety of organs and tissues, particularly liver, lung, and the large arteries. Heparin is a polymer of alternating α -D-glucosamine and hexosamine residues joined by (1,4) glycosidic linkages. When
 10 glycosaminoglycans are synthesised in nature, typically they are conjugated to a central protein core. However, preferably the glycosaminoglycans employed in the invention will lack such a central core. Typically, glycosaminoglycan preparations will lack a core and may be employed or, if present, the core can be removed. Commercially available preparations of glycosaminoglycans will usually lack the
 15 core and may be employed.

Heparin is clinically used as an anti-coagulant, where it is thought to exert its effects through interaction with anti-thrombin III (AT-III) and heparin co-factor II and other coagulation factors. Typically the heparin will retain some anticoagulant activity i.e. be able to increase clotting time in an individual. Thus preferably the
 20 heparin will be able to bind anti-thrombin III (AT-III) and/or heparin co-factor II (HCII) and hence inhibit clotting. Preferably it will be able to form a complex with AT-III, heparin and a clotting factor. However, in some embodiments a heparin which lacks anti-coagulant activity or which has reduced anti-coagulant activity may also be employed. Thus the heparin may have been modified so that it has from 0 to
 25 80%, preferably from 5 to 60%, more preferably from 10 to 40% and even more preferably from 10 to 50% of the activity of the unmodified form or in comparison to unmodified heparin. Other glycosaminoglycans, in particular dermatan sulphate, also possess anti-coagulant activity. Preferably, therefore, the glycosaminoglycans and their derivatives employed will retain some anti-coagulant activity, as discussed
 30 above for heparin and its derivatives.

The glycosaminoglycan will be typically be administered via inhalation,

intranasally. In installation preferably it will be in a form suitable for administration via such a route. In particular, the glycosaminoglycan may be in a form suitable for installation and/or installation.

5 *Subject treatment*

The present invention provides a method of treating CAL comprising administering to a subject with CAL a glycosaminoglycan, or a salt thereof, which glycosaminoglycan or salt has an average molecular weight of from 8 to 40 kilodaltons. The glycosaminoglycan or salt used in such methods, the route of
10 delivery and any of the other parameters of the method and subject being treated may be the same as described herein for any of the other embodiments of the invention.

The embodiments and methods of the invention preferably induce an improvement in the condition of the subject. The medicaments and methods may therefore be used to manage a patient suffering from, or prone to, CAL. They may
15 prevent, slow down, improve or cure the condition. They may slow down or arrest the progressive deterioration characteristic of CAL or in some cases even cause some reversal of the deterioration. They may prevent, reduce or reverse one or more of the symptoms and manifestations associated with CAL. They preferably will also increase the feeling of well being in the subject and their quality of life.

20 A medicament or method of the invention preferably reduces, eliminate or at least prevents further increase in one or more of:

- the elevated decline in forced expiratory volume (FEV₁);
- excessive secretion;
- inflammation; and/or
- 25 - damage to the structure of the lung.

Thus, in view of the medicaments of the invention may also mean that the ratio of FEV₁ to the decline further, or is improved. For example, the ratio may be closer to that expected in a healthy subject.

The medicaments and methods may reduce mucus plugging. They may have
30 mucolytic activity and/or reduce secretion and increase expectoration of mucus. The medicaments and methods of the invention may facilitate the clearance of mucus and,

due to their mucolytic activity, prevent or ameliorate detrimental effects associated with elevated mucus levels and increased viscosity in CAL. The medicaments and methods of the invention may result in a reduction of mucus viscosity of, for example, at least one fold, preferably two fold and more preferably five fold. In some cases the decrease in viscosity may be from 10 to 80%, preferably from 20 to 60% and even more preferably from 30 to 40%.

Mucolytic activity refers to the reduction of viscoelasticity (viscosity) of mucus. Mucolytic activity may be determined by any of several different methods known in the art, including sputum compaction assay (see for example WO 94/10567) assays using a torsion pendulum (Janmey, J. Biochem. Biophys. Methods 22:41-51, 1991) or other suitable rheological methodologies. The decrease in mucus viscosity brought about by the medicaments and methods of the invention may be referred to as mucokinesis.

The medicaments and methods of the invention may reduce the breakdown of the structure of the lung, such as the degradation of elastin in the airways and in the aveolus and hence the loss of lung elasticity. They may reduce or prevent the collapse of portions of the lung and/or the development of enlarged airspaces in which air can become trapped. The medicaments may prevent or reduce any of the pathological changes associated with CAL outlined herein. In particular, they may prevent the onset of a pathological change. They may also prevent, or delay, the onset of a more severe pathological change.

The medicaments and methods of the invention may typically reduce the decline in FEV₁ by from 10 to 100%, preferably from 20 to 80%, more preferably from 30 to 60% and even more preferably from 40 to 50%. They may reduce the annual decline in FEV₁ by from 10 to 100 ml, preferably from 20 to 60 ml and even more preferably from 30 to 40ml per year. In some cases on treatment the subject will display an improvement of FEV₁ so that FEV₁ is from 25 to 100%, preferably from 40 to 100% more preferably from 60 to 100% and even more preferably from 80 to 100% of the predicted value.

The methods and medicaments of the invention may reduce inflammation and the influx of inflammatory cells to the airways and lungs. They may also

ameliorate the effect of the inflammatory cells present in the lung. Thus differential cell counts in sputum and/or lavages from the subject may display normal levels, or less elevated levels, of inflammatory cells such as lymphocytes, macrophages and/or neutrophils and in particular of neutrophils.

- 5 They may reduce migration of inflammatory cells such as neutrophils to the airway in the lung. They may also reduce one or more of neutrophil functional characteristics such as priming, activation, chemotaxis, extravasation, degranulation, respiratory burst, phagocytosis, apoptosis and/or necrosis. This may also mean that there is a reduced amount of neutrophil proteins in the lung and
- 10 reactive oxygen species. There may also be reduced levels of inflammatory mediators in sputum following treatment and in particular of IL-8 levels. They may also cause changes in the lung such as the down regulation of adhesion molecules so that inflammatory cells and in particular neutrophils cannot enter the lung, do so less readily or do so in reduced numbers.
- 15 The treatments of the invention may eliminate, delay the onset, or reduce the severity of any of the symptoms and features of CAL mentioned herein.

Administration and Formulation

- The active ingredients of the present invention may be prepared by formulating
- 20 the glycosaminoglycan, glycan or salt with a standard physiologically, and in particular pharmaceutically, acceptable carrier and/or excipient as is routine in the pharmaceutical art. The exact nature of the formulation will depend upon several factors including the particular glycosaminoglycan employed and the desired route of administration. Such types of formulation are fully described in Remington's
- 25 *Pharmaceutical Sciences*, 19th Edition, Mack Publishing Company, Eastern Pennsylvania, U.S.A., the disclosure of which is included herein of its entirety by way of reference.

- The amount of glycosaminoglycan to be administered will normally be determined by a physician or pharmacist upon a number of factors such as the nature of the
- 30 condition being treated, the condition of the patient. The dose of glycosaminoglycan administered may, for example, be from 0.01 mg to 5g, preferably from 0.1 mg to

2.5g, more preferably from 1 mg to 1g, even more preferably from 10 mg to 500mg, still more preferably from 50 mg to 250 mg and even more preferably from 100 mg to 200 mg. These doses will typically be given once, twice or three times a day and will preferably be given once or twice a day and more preferably will be given twice a day.

For glycosaminoglycans and derivatives thereof and salts of either, dose will typically be in the range of from 1 to 10,000 units per/kg body weight, preferably 100 to 10,000 units per/kg body weight, more preferably from 200 to 5000 units/kg body weight, even more preferably from 500 to 2000 units per/kg, and still more preferably from 1,000 to 1,500 units per/kg. A unit of heparin activity (United States Pharmacopeia) is defined as the amount of heparin that prevents 1ml of citrated sheep plasma from clotting in one hour after adding 0.2 ml of 1% CaCl_2 . These doses will typically be given once, twice or three times a day and will preferably be given twice a day.

The duration of treatment may typically be from two weeks, a month, six months, a year or more. In many cases the subject will remain on the medicaments of the invention for a long time or for extended periods. This may in particular be the case where the subject does not stop smoking or continues to be exposed to the chemical pollutant thought to be the causative agent. This may also be the case where the subject has a genetic predisposition to developing CAL and most likely will need it indefinitely. The treatment schedule may also be coordinated so that at times when the CAL increases in severity such as times, or periods, of increased breathlessness and/or inflammation, that the dose of glycosaminoglycan given is elevated or these may be given in addition that glycosaminoglycan is administered. The medicament may be given prior to, during or after physical exertion and may typically be given as an aid to physical exertion. The medicament may be given during infections or when an infection is suspected.

Typically the medicaments of the invention are administered via inhalation and/or intranasally. This administration may typically be via the mouth or nose. In an especially preferred embodiment, the medicaments of the invention are suitable for administration by inhalation and are administered via that route. The medicaments may alternatively may be, suitable for administration via intranasal or other methods for formulating and preparing medicaments to be

administration, inhalation, intranasal and intramuscular are well known in the art and are not novel in the present invention.

For intranasal therapy the medicament may be in a solution useful for administration by liquid aerosol, metered dose inhalers, or in a form suitable for a dry powder inhaler. The medicament may be present in a blister pack or breakable capsule.

In some preferred embodiments, the medicaments of the present invention may be formulated as aerosols. The formulation of pharmaceutical aerosols is routine to those skilled in the art, see for example, Solkara, J. in Remington (*supra*). The agent may be formulated as solution aerosols, dispersion or suspension aerosols of dry powders, or as liquid or semisolid preparations. The aerosol may be delivered using a nebuliser or metered dose inhaler known to those skilled in the art. The aerosols may be applied to the upper respiratory tract, for example by nasal inhalation, or to the lower respiratory tract or to both. The glycosaminoglycan may be delivered using liposomes or other particle delivery methods. Liposomes, particularly cationic liposomes, are well known in cancer formulations.

The formulations for use in accordance with the present invention, may include, in addition to the active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. In particular they may include a pharmaceutically acceptable excipient. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration. Suitable pharmaceutical carriers are described in Remington (*supra*).

The medicaments of the present invention may be delivered by any device adapted to deliver the therapeutic compositions into the upper and/or lower respiratory tract. In some preferred embodiments, the devices of the present invention may be metered-dose inhalers. The devices may be adapted to deliver the therapeutic compositions of the invention in the form of a finely dispersed mist of liquid or dry particles. The device may use a piezo electric effect or ultrasonic vibration to disperse powder attached on a surface such as a tape in order to generate

mist suitable for inhalation. The devices may use any propellant system known to those in the art including, but not limited to, pumps, liquefied-gas, compressed gas and the like.

In cases where the glycosaminoglycan is administered in the form of particles or droplets, the particle/droplet size and/or other properties of the particle/droplet may be chosen to ensure that the particles are delivered to a particular region of the respiratory tract. For example, they may be designed to reach only the upper or lower parts of the respiratory tract. In cases where the glycosaminoglycan, salt or other compound are delivered in an aqueous form preferably the solution will be isotonic to help ensure effective delivery to the subject. In particular, particles with a diameter of $10\text{ }\mu\text{m}$ are thought to be effective in reaching the lower parts of the respiratory tract and hence may be employed where such a site is the desired target for the medicament. In embodiments, where it is desired to deliver the medicament to the lower parts of the respiratory tract, such as alveoli for example, the diameter of the particles administered may be less than $10\text{ }\mu\text{m}$, preferably less than $8\text{ }\mu\text{m}$, more preferably less than $6\text{ }\mu\text{m}$ and even more preferably less than $4\text{ }\mu\text{m}$. In a preferred embodiment the particles may have a diameter of $3\text{ }\mu\text{m}$ or less and more preferably may have a diameter of $2\text{ }\mu\text{m}$ or less. In an especially preferred embodiment the particles will have a diameter of from 3 to $5\text{ }\mu\text{m}$. In some cases the particles administered may have a diameter of 100 nm , preferably less than 500 nm , more preferably less than 250 nm and still more preferably less than 100 nm in diameter. The particles may be particles of solid matter or droplets of solutions and suspensions.

It will be appreciated that it may be necessary to penetrate to a specific part of the respiratory tract with a known location and hence the particle size can be chosen to suit the target area. Techniques such as nebulising may be used to produce the very small particles necessary, in some cases the desired part of the respiratory tract may be the upper respiratory tract and hence larger particles sizes may be employed. The density of the particles and their shape may also be chosen to facilitate their delivery to the desired location.

The embodiments of the invention may take a variety of forms. They may be

in the form of powders, microspheres, solutions, suspensions, gels, nanoparticles, emulsions, suspensions or other emulsions. The liquids present may be in or dissolved in solvents such as a CFC or HFA. In the case of solutions and suspensions, these may be aqueous or involve solutions other than water.

Devices of the present invention typically comprise a container with one or more outlets through which the flow of the therapeutic composition travels and an actuator for controlling the flow. Suitable devices for use in the present invention may be found, for example, in Remington's *(supra)*. The devices suitable for

administration of the compositions of the invention include inhalers and nebulisers such as those typically used to deliver steroids to asthmatics. In some cases, for example where the subject is a child, a spacer may be used in conjunction with the inhaler to help ensure effective delivery.

The medicaments of the present invention may be delivered by any device

adapted to introduce one or more therapeutic compositions into the upper and/or lower respiratory tract. In some preferred embodiments, the devices of the present invention may be metered-dose inhalers. The devices may be adapted to deliver the therapeutic compositions of the invention in the form of a finely dispersed mist of liquid, foam or powder. The device may use a piezoelectric effect or ultrasonic

vibration to dislodge powder attached on a surface such as a tape in order to generate mist suitable for inhalation. The devices may use any propellant system known to those in the art, but are not limited to, for example, liquefied-gas, compressed gas and foam.

Devices of the present invention typically comprise a container with one or more outlets through which the flow of the therapeutic composition travels and an actuator for controlling the flow. Suitable devices for use in the present invention may be found, for example, in Remington's Pharmaceutical Sciences (*supra*). The devices suitable for administering the medicaments of the invention include inhalers and nebulisers such as those typically used to deliver steroids to asthmatics. In some cases, for example where the subject is a child, a spacer may be used to facilitate effective delivery of the medicament from the inhaler.

Various designs of inhalers are available commercially and may be employed to deliver the medicaments of the invention. These include the Accuhaler, Aerohaler, Aerolizer, Airmax, Autohaler, Clickhaler, Diskhaler, Easi-breathe inhaler, Fisonair, Integra, Jet inhaler, Miat-haler, Novolizer inhaler, Pulvinal inhaler, Rotahaler, 5 Spacehaler, Spinhaler, Synchroner inhaler and Turbohaler devices. A number of formulation techniques which produce particularly desirable particles are known in the art and may be employed. For example the nanocrystal, pulmosol and pulmosphere technologies may be employed.

In some cases the medicaments may be administered via installation. In such 10 cases, typically the medicament will be in liquid form and will be administered via an artificial airway such as, for example, an endotracheal tube. The liquid will typically be drawn up into a syringe and then expelled through the artificial airway into the respiratory tract of the subject. Installation is often used in an emergency context. In many cases it may be used where the subject has a relatively advanced 15 form of CAL and has been admitted to hospital.

The medicaments of the invention may also be administered via the nose. Again, suitable methods, formulations and devices are known in the art for intranasal administration and may be employed. In cases where the medicament is administered via the nose the actual target site may be the nasal mucus membranes or alternatively 20 some other part of the respiratory tract. The medicament will be typically formulated and administered accordingly. In cases where the medicament is to be administered via the nose it may be, for example, in the form of nasal spray. The spray may be administered, for example, using an atomizer or nebulizer. In some cases the medicament may be in the form of nasal drop. These may be administered using a via 25 a medicine dropper. For further discussion on nasal dosage forms see Remington's Pharmaceutical Sciences (*Supra*). Medicaments administered via the nose may include pH adjusters, emulsifiers or dispersing agents, preservatives, surfactants, gelling agents, or buffering agents. Most preferably the nasal dosage form will be isotonic with nasal secretions.

30 The medicaments may include various constituents to optimise their suitability for the particular delivery route chosen. The viscosity of the medicaments

may be maintained at a desired level using a pharmaceutically acceptable thickening agent. Thickening agents that can be used include methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof. The concentration of the thickening agent will depend upon the agent selected and the viscosity desired.

In some embodiments, and in particular where intranasal delivery is to be used, the medicaments may comprise a humectant. This may help reduce or prevent drying of the mucous membrane and to prevent irritation of the membranes. Suitable humectants include sorbitol, mineral oil, vegetable oil and glycerol; soothing agents; membrane conditioners; sweeteners; and combinations thereof.

The medicaments may comprise a surfactant. Suitable surfactants include non-ionic, anionic and cationic surfactants. Examples of surfactants that may be used include, for example, polyoxyethylene derivatives of fatty acid partial esters of sorbitol anhydrides, such as for example, Tween 80, Polyoxyl 40 Stearate, Polyoxyethylene 50 Stearate, fusicates, bile salts and Octoxynol.

Kits

A kit of the invention comprises:

- a glycosaminoglycan or a physiologically acceptable salt of either thereof, which glycosaminoglycan or salt has an average molecular weight of from 8 to 40 kilodalton;
- instructions for administering the glycosaminoglycan or salt to a subject suffering from CAL; and
- packaging.

The instructions may provide information on recommended dosages, times of administration, how to administer the glycosaminoglycan and other advice. The kit may also comprise means to administer the glycosaminoglycan such as any of those discussed herein.

The following Examples illustrate the invention.

Example 1: Barrier Assay on Victor Fluorometer

Sputum samples were thawed at room temperature and homogenised using a syringe (no needle). Samples were split and portions (0.12-0.20 g) weighed in small round-bottomed tubes. Tubes were centrifuged in a microfuge to sediment sputum.

- 5 Mucolytic was added to sputum on a 10% volume to weight basis (e.g. 13 μ l/0.13 g sputum). The mucolytic was therefore diluted by a factor of 10 upon addition to sputum. The mucolytic enzymes were DNase (at a concentration of 2.9 μ g/ml of a stock of activity 2500 units per mg) and unfractionated heparin (at a concentration of 10 mg/ml of a stock of 160 USP/mg – a sodium heparin salt isolated
- 10 from porcine intestinal mucosa from Calbiochem, catalogue number 375095, was employed). A control portion of sputum was treated with PBS on a 10% volume to weight basis. A further control was treated with dextran sulphate, which is not a glycosaminoglycan, at a concentration of 10 mg/ml (a sodium salt of dextran sulphate obtained from Sigma, catalogue number D7037, was employed). The
- 15 mucolytic was then thoroughly mixed with the sputum by vortexing and passing up and down a 1.5 ml microcentrifuge tube.

- The lower wells of a Transwell chamber contained 25 μ l of phosphate buffered saline (PBS) and this was separated from the upper wells by an 8 μ m pore-size polycarbonate filter (placed shiny side down). The upper section of the chamber
- 20 was placed on top of the filter and secured down tightly. Carboxylate-modified fluorescent beads (CF-5011, Molecular Probes) were sonicated for 5 mins to disperse them and then added to the sputum samples in a 1.5 ml tube, e.g. 150 μ l beads added to 0.15 g sputum sample. The mixture was vortexed to mix and 20 μ l of each sample added to the upper wells of the chamber. Each sample was added to the chamber in
- 25 quadruplicate. The chamber was centrifuged for 5 mins at 1000 rpm to remove air bubbles, then placed inside a black plastic bag and incubated in a humid chamber at 37°C for 16 hours.

- After incubation, the chamber was centrifuged for 5 mins at 1000 rpm to force sedimented beads to the bottom of the lower wells. The upper chamber and gasket
- 30 were removed in order to minimise leakage. The contents of the lower wells

(25µl) were transferred to a Micro-NUNC plate and 175µl PBS added per well. The plate was read using a Microfluorometer with the 'Fluorespheres' protocol (excitation 485nm, emission 525nm).

The results obtained are shown in the top panel of Figure 1. The mucolytic effects of heparin were smaller and less variable than those of DNase. A Bonferri multiple comparison test showed that the effect of 10 mg/ml heparin was significantly greater than that of the PBS control ($P < 0.01$). However, the effects of DNase and 10 mg/ml deoxycholate (negative control) were not significantly different from deoxycholate or PBS control ($P > 0.1$).

The effect of chondroitin sulphate on microsphere transport was also assessed using the same method as those described above. A 70:30 mixture of chondroitins A and C was used (16 mg/ml) as all of the chondroitins from bovine trachea, obtained from the supplier (Sigma, C-8529, was employed). A control containing sputum treated with PBS alone was again run. The results obtained are shown in the bottom panel of Figure 1. Chondroitin sulphate was found to significantly increase transport of the microspheres.

Example 3: Atomic Force Microscopy (AFM) imaging of DNA

Calf thymus DNA (0.1 mg/ml) was treated with heparin at various concentrations (0.1, 1, and 100 µg/ml). Solutions were prepared in 0.2 µm filtered, DNase and RNase free water. 10 µl of sample was added to freshly cleaved ruby muscovite microcrystals prior to AFM imaging. AFM was performed in air under ambient conditions using a TopoMatrix TM112000 Scanning Probe Microscope (Thermo Microscopes, Bicester, UK) with a 70/70/12 mm tripod piezoelectric transducer for topography measurements. In constant mode, were made using a "V"-shaped silicon cantilever (length 200 µm, nominal spring constant (K) 0.21 N/m) (from Microscopica, Thermal Microscopes, Santa Clara, California, USA) bearing an integrated piezoelectric probe tip.

The results are shown in Figures 2 and Figure 3. In Figure 2, Panel A = 0.1 mg/ml DNase and Panel B = 0.1 mg/ml DNA + 0.1 µg/ml heparin. The addition of heparin is seen to alter the structure of the DNA network, increasing the

average pore size from 180 (SD 30) μm to 780 (SD 150) μm .

Figure 3 shows results with lower concentrations of heparin. Panel A shows the results for untreated DNA; Panel B for DNA treated with 0.1 $\mu\text{g/ml}$ heparin; Panel C for DNA treated with 1 $\mu\text{g/ml}$ heparin; and Panel D for DNA treated with 10 $\mu\text{g/ml}$ heparin.

Example 3: Patient Assessment

- A female patient, age 71 years, with ALD (an ex-smoker, ceased 6 years ago) inhaled undiluted heparin at a dose of 50,000 units, twice a day for 14 days.
- 10 Although there was no change in spirometry over this short period, there was a marked improvement of the symptoms of cough, and improved clearance of sputum.

average pore size from 180 (SD 30) μm to 780 (SD 150) μm .

Figure 3 shows results with lower concentrations of heparin. Panel A shows the results for untreated DNA; Panel B for DNA treated with 0.1 $\mu\text{g/ml}$ heparin; Panel C for DNA treated with 1 $\mu\text{g/ml}$ heparin; and Panel D for DNA treated with 10 $\mu\text{g/ml}$ heparin.

Example 3: Patient Assessment

A female patient, age 81 years, with ALD (an ex-smoker, ceased 6 years ago) inhaled unfractionated heparin at a dose of 50,000 units, twice a day for 14 days.

10 Although there was no change in spirometry over this short period, there was a marked improvement in symptoms of cough, and improved clearance of sputum.

CLAIMS

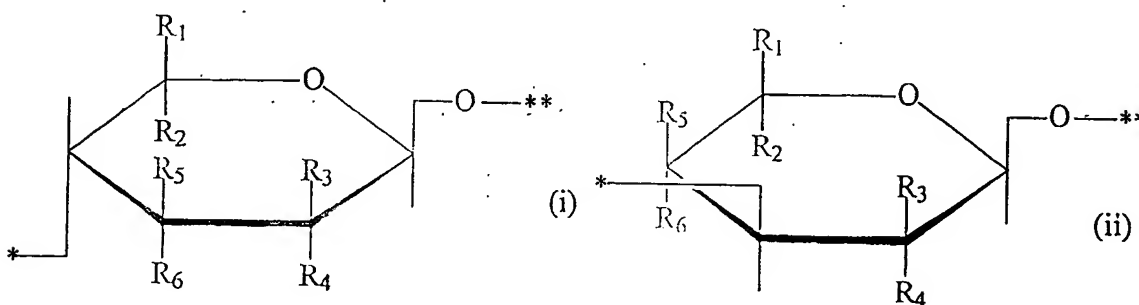
1. Use of a glycosaminoglycan or a physiologically acceptable salt thereof in
 5 the manufacture of a medicament for treating chronic airflow limitation (CAL)
 wherein the said glycosaminoglycan or salt has an average molecular weight of from
 8 to 40 kilodaltons.

2. Use according to claim 1, wherein the glycosaminoglycan or the
 10 physiologically acceptable salt comprises repeating disaccharide units of general
 formula (1)



wherein :

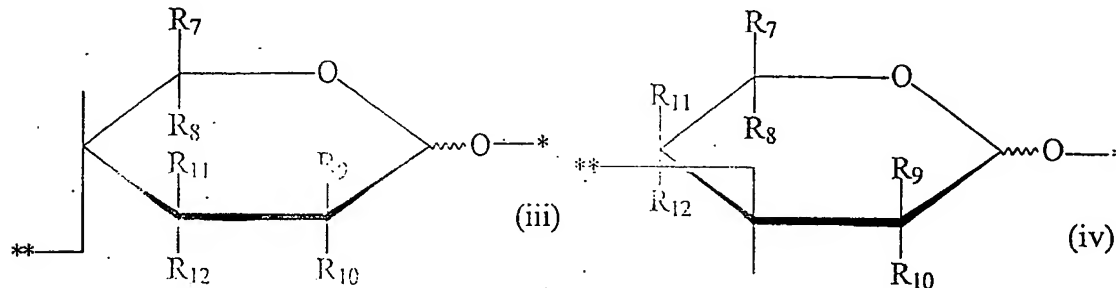
15 each A is the same or different and represents a moiety of formula (i) or (ii)



wherein:

- 20 - one of R_1 and R_2 is hydrogen, and the other is $-\text{CO}_2\text{H}$, $-\text{SO}_3\text{H}$ or $-\text{CH}_2\text{OR}$
 wherein R is hydrogen or $-\text{SO}_3\text{H}$;
- one of R_3 and R_4 is hydrogen, and the other is $-\text{OR}$ wherein R is hydrogen or $-\text{SO}_3\text{H}$;
- one of R_5 and R_6 is hydrogen, and the other is $-\text{OH}$;
- 25 - * represents a direct bond to an adjacent hydrogen atom or B moiety; and

- ** represents a direct bond to an adjacent B moiety;
- each B is the same or different and represents a moiety of formula (iii) or (iv)



5 wherein:

- one of R_7 and R_8 is hydrogen and the other is $-\text{CH}_2\text{OH}$ or $-\text{CH}_2\text{OSO}_3\text{H}$;
- one of R_9 and R_{10} is hydrogen and the other is $-\text{NHAc}$, $-\text{NH}_2$ or $-\text{NHSO}_3\text{H}$;
- one of R_{11} and R_{12} is hydrogen and the other is $-\text{OH}$ or $-\text{OSO}_3\text{H}$;

10 indicates a bond in either stereochemical orientation;

- * represents a direct bond to a hydrogen atom or an adjacent A moiety;
- ** represents a direct bond to an adjacent A moiety.

or a physiologically acceptable salt thereof.

15 3. Use according to claim 1 or 2, wherein the glycosaminoglycan is a heparin or a derivative thereof.

4. Use according to any one of the preceding claims, wherein the sodium salt of heparin or heparin sulphate is used.

20

5. Use according to claim 1 or 2, wherein the glycosaminoglycan is chondroitin sulphate A, chondroitin sulphate C, chondroitin sulphate D, chondroitin sulphate E, hyaluronic acid, keratan sulphate, heparan sulphate or a derivative of any thereof.

25 6. Use according to claims 5, wherein the glycosaminoglycan is chondroitin sulphate A, chondroitin sulphate C or heparan sulphate or a derivative of any thereof.

7. Use according to any one of the preceding claims, wherein the
glycosaminoglycan or salt has an average molecular weight of from 12 to 18 kd.
- 5 8. Use according to any one of the preceding claims, wherein the
glycosaminoglycan or salt has anti-coagulant activity.
9. Use according to any one of the preceding claims, wherein the
10 glycosaminoglycan or salt has not been subjected to fragmentation and/or
depolymerisation.
10. Use according to any one of the preceding claims, wherein the medicament is
administered via inhalation, intranasally, and/or via installation.
- 15 11. Use according to any one of the preceding claims, wherein the medicament is
for treating a subject who has a FEV₁ which is from 20 to 50% of the predicted value
for an equivalent subject not suffering from CAL.
- 20 12. Use according to any one of the preceding claims, wherein the medicament is
for treating a human subject.
13. Use according to claim 12, wherein the subject is, or has been, a smoker.
- 25 14. Use according to any one of claim 1 to 11, wherein the medicament is for
treating a non-human animal.
15. Use according to claim 14, wherein the animal is a horse, dog, cat, cow or
bull.
- 30 16. A method of treating CAL comprising administering to a subject suffering

from CAL an effective amount of glycosaminoglycan or a physiologically acceptable salt thereof as defined in any one of claims 1 to 9.

17. A method according to claim 16, wherein the glycosaminoglycan or salt is
5 delivered via inhalation, intranasally or via installation.

18. An agent for treating CAL comprising glycosaminoglycan or a
physiologically acceptable salt thereof as defined in any one of claims 1 to 9.

10 19. A kit comprising:
- a glycosaminoglycan or a physiologically acceptable salt thereof as
defined in any one of claims 1 to 9;
- instructions for administering the glycosaminoglycan or salt to
a subject suffering from CAL; and

15 - a syringe.

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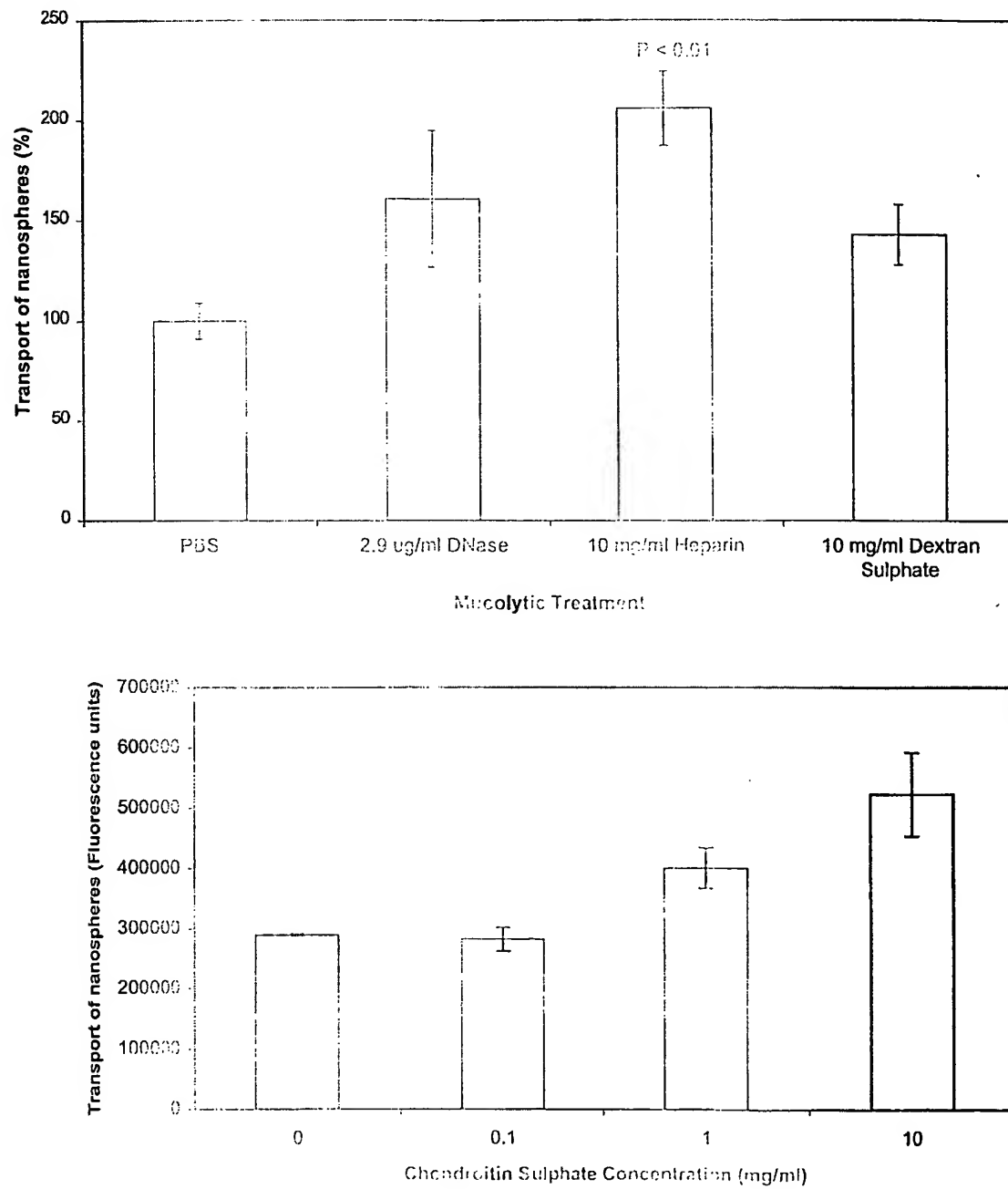


FIGURE 1.

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FIGURE 2

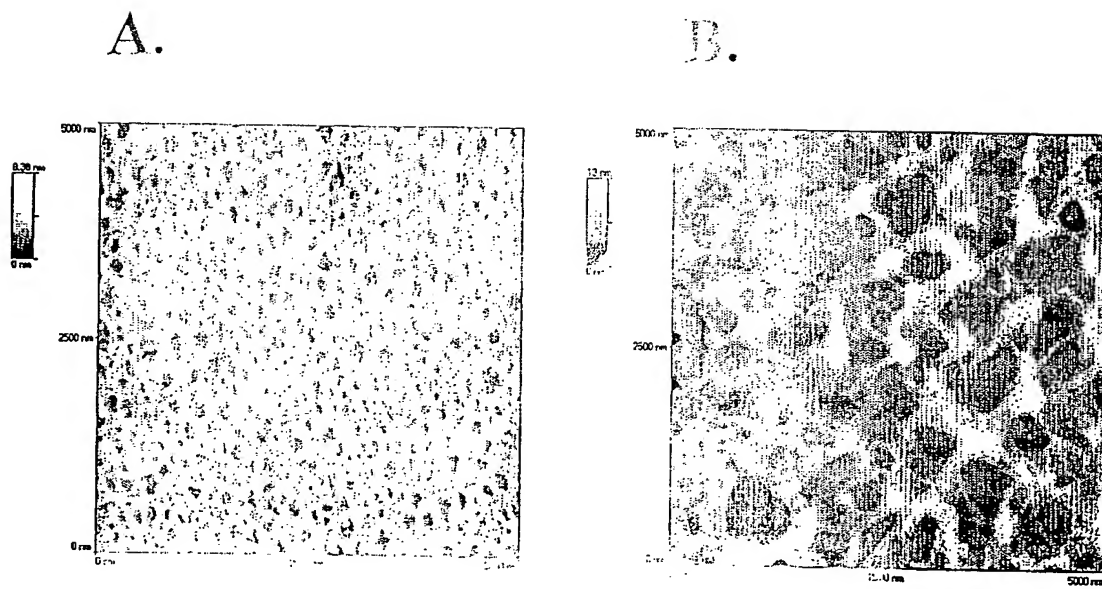
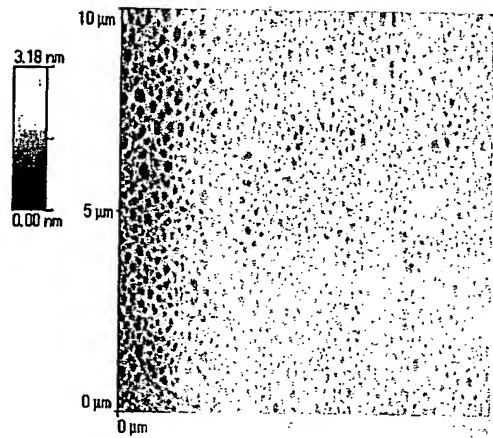
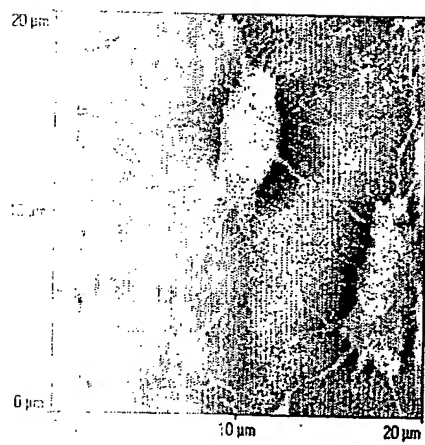
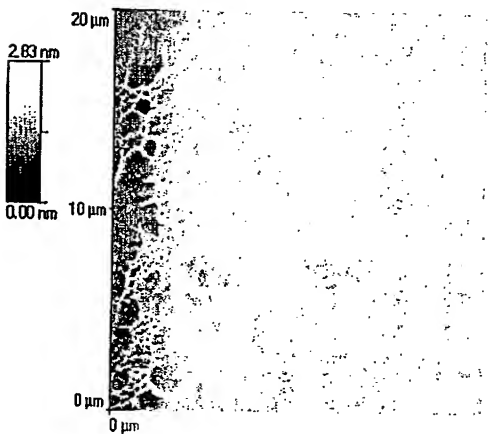
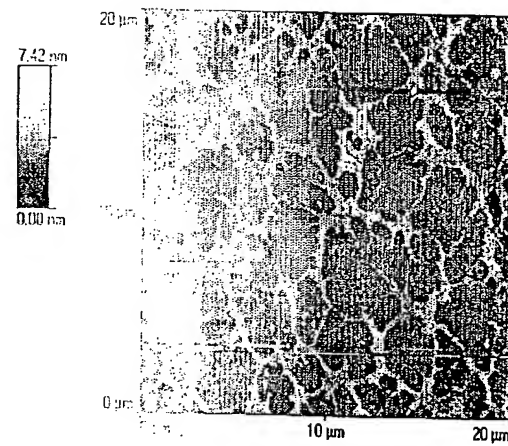


FIGURE 3

A.



B.



C.

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INTERNATIONAL SEARCH REPORT

Application No

03/00663

A. CLASSIFICATION
IPC 7 A61K A61K31/727 A61P11/00 A61P9/72

According to International classification and IPC

B. FIELDS SEARCHED

Minimum documentation by classification symbols)

IPC 7 A61K

Documentation searched other than the extent that such documents are within the fields searched

Electronic data base Name of data base and, where possible, the search

EPO-Internal Data IS, EMBASE, CHEM ABSTRACT

C. DOCUMENTS CITED

Category *	Citation of documents with indication of the relevant passages	Relevant to claim No.
P, X	WO 01/49512 (2002-04-25) TECHNICAL FIELD the whole document	1-19
P, Y	claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100	1-19
Y	WO 01/49512 (2002-04-25) 11 the whole document * page 10, paragraph 1 page 10, paragraph 1 page 10, paragraph 1 page 10, paragraph 1 claim 1	1-19

-/--



Further documents cited



Patent family documents cited in annex.

* Special categories

- *A* document defined as prior art, considered to be
- *E* earlier document filing date
- *L* document which is cited in the citation or other
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- *P* document published later than the

T later document published after the international filing date or priority date but which is cited with the application but not cited to understand the principle or theory underlying the invention

X document of prior art which cannot be considered to be taken alone to give an inventive step when the document is considered in the context of the other documents cited in the application

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Date of the actual date of filing

22 May 2002

Date of mailing of international search report

23/05/2002

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THE FBI REPORT

C.(Continuation) 1.

Category *

Citati:

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2. rel. "passages"

Relevant to claim No.

A

ANS: C RATIONALE: COPD is a chronic lung disease that causes difficulty breathing.

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A ... MIT. SCFD, 'Online!

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Re: [redacted] [redacted]

<http://www.ck12.org/Intro/definition.htm>

1. "1955-56" 5-22!

21.

Application No.
PCT/GB 03/00663

INTERNATIONAL SEARCH REPORT

Box I Observations on certain claims found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos. 1-3 because they are not drafted in accordance with the requirements to be searched by this Authority, namely:
see Form PCT/ISA/210
2. ☐ Claims Nos. 4-6 because they are not drafted in accordance with the requirements to be searched by this Authority, namely: they do not comply with the required requirements to such an extent that they are not drafted out, specifically:
3. ☐ Claims Nos. 7-9 because they are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations on certain claims found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all search fees were timely paid by the applicant, this International Search Report covers all searchable claims. No additional search fees were paid, and the Authority did not invite payment of any additional search fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos. 1-3.
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the claims for which fees were paid, specifically claims Nos. 1-3.

Remark on Protest:

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Although the above information is directed to a method of treatment of the human/animal, no actual treatment has been carried out and based on the alleged information, the method is not a valid/operation.

Rule 39 1. A method or process for treatment of the human or animal body by therapy

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PCT/US 03/00663

 Internal
 Application No
 PCT/US 03/00663

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			EP 5000111 A1 11-02-1999
			EP 5000111 A1 27-02-2001
			EP 5000111 A1 28-01-1999

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